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Bluetongue and epizootic hemorrhagic disease viruses: recent developments with these globally re-emerging arboviral infections of ruminants

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Bluetongue (BT) and epizootic hemorrhagic disease (EHD) are globally re-emerging diseases of domestic and wild ruminants, respectively caused by BT virus (BTV) and EHD virus. Both viruses are transmitted by hematophagous midges; however, newly recognized BTV serotypes may be transmitted horizontally without requirement for any biological vector. The global range of these viruses and/or their associated diseases have changed remarkably in recent years, most notably with the invasion of Europe by multiple serotypes of BTV since 1998. Although not zoonoses, the unanticipated emergence of BT and EHD in several different areas of the world provides a uniquely sobering and unambiguous reminder of the potential consequences of climate change on the distribution and severity of vector-borne diseases. Recent experiences with these viruses have also emphasized the need for effective, DIVA-compatible vaccines to combat anticipated future incursions, as existing vaccines have serious inherent deficiencies.

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Introduction

Bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV) are closely related arboviruses that are classified in the genus *Orbivirus* within the subfamily Sedoreovirinae of the family Reoviridae [1•,2•,3]. Both viruses are transmitted to wild and domestic ruminants by certain species of hematophagous *Culicoides* midges. Whereas, BTV is the cause of significant economic loss to ruminant livestock production, especially of sheep, EHDV is most significant as a pathogen of ruminant wildlife, notably white-tailed deer (*Odocoileus virgnianus*). BTV and EHDV are genetically diverse, with multiple serotypes and myriads of genetically distinct strains of each virus [4,5•,6,7•,8,9]. Genetic diversity within these virus species occurs as a consequence of both genetic drift and genetic shift; genetic shift occurs as a consequence of reassortment of viral gene segments during co-infections of either animals (ruminants) or insect vectors (*Culicoides* midges) with more than one virus serotype or strain, whereas genetic drift occurs as a consequence of polymerase 'infidelity' during replication of the virus leading to a viral quasispecies with subsequent founder effect of specific genetic variants in either insect or animal hosts. The individual species of *Culicoides* midge that serve as vectors of these viruses differ between regions of the world, as do the serotypes and strains of BTV and EHDV that occur within each global 'episystem' [4,9,10•,11]. Thus, the respective global ranges of BTV and EHDV infections are similar but certainly not identical. Furthermore, there have been profound recent changes in the global distribution and biological behavior of both BTV and EHDV. Given that the epidemiology, pathogenesis and other features of BTV and EHDV infections have recently been reviewed [1•,2•,3], the purpose of this article is to summarize recent novel developments regarding characterization of the global ecology and biological behavior of these two viruses.

Bluetongue

Expansion of global range

The global range of BTV infection has changed remarkably with the advent of the new millennium, notably at the traditional upper and lower limits of the virus' global range (approximately 50′ North and 35′ South) [2•]. Climate change is proposed to have contributed this expansion in the traditional global range of BTV as a consequence of its impact on the distribution of vector-competent species of

midges as well as the vectorial capacity of populations of midges already resident in different regions of the world (including those not previously thought to be competent vectors) [12•]. Notable recent changes in the global distribution of BTV include: 1. Incursion of multiple serotypes of BTV into Europe [2•,12•]. Before 1998, only portions of Europe within the Mediterranean Basin were subject to incursions of BTV, and these were transient and involved only single virus serotypes; 2. Incursion since 1999 of multiple novel BTV serotypes into the southeastern United States from the adjacent Caribbean episystem [2•,13]; 3. First detection of BTV in Ontario, Canada, in 2015 as a result of northern spread of the virus from the United States [14]; 4. Extension of BTV into Victoria, Australia, in 2017, which is farther south than the virus' traditional distribution on that continent [15]; 5. Increasing detection of multiple BTV serotypes in South America and Asia, including countries such as South Korea that had not previously documented such infections [16–18].

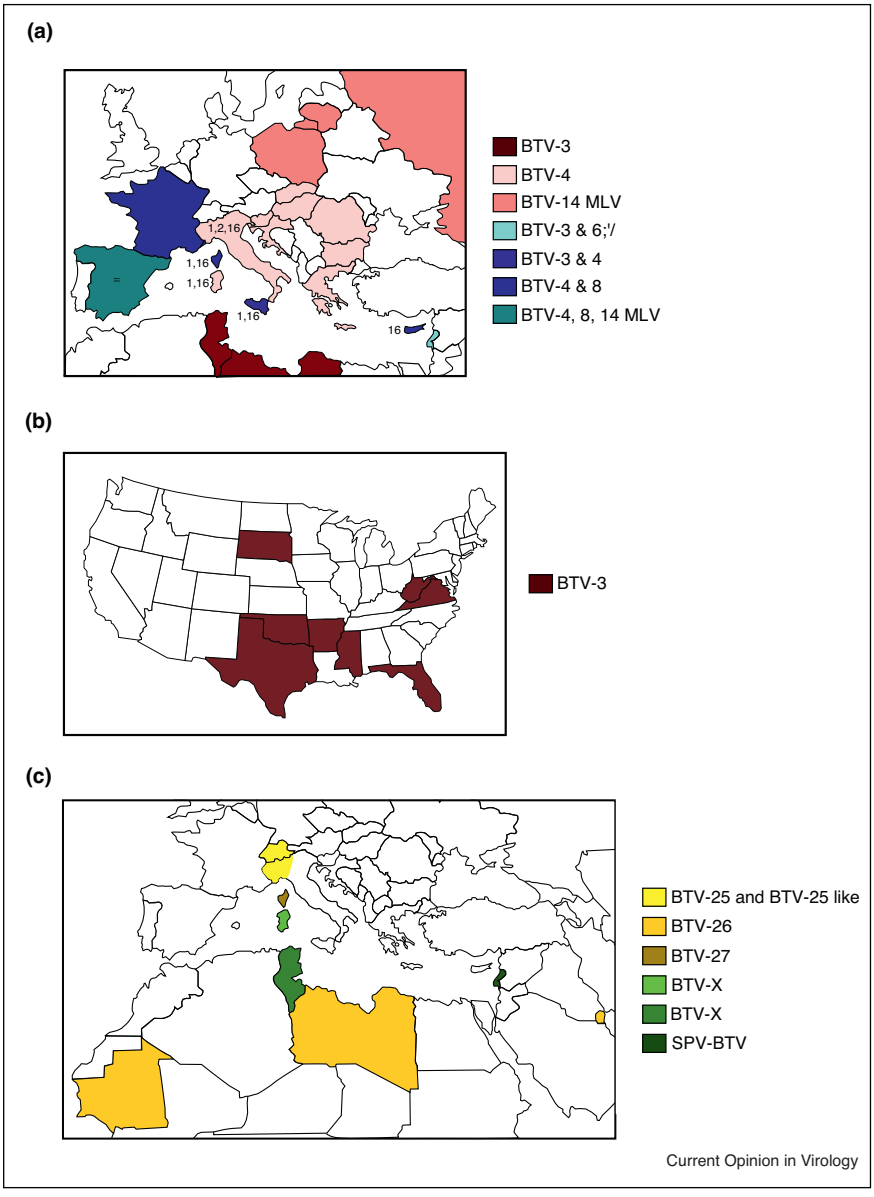
Notable recent findings regarding the global epidemiology of BTV infection include (see Figure 1):

1 *Reemergence of BTV serotype 8 in France*. BTV serotype 8 (BTV-8) first emerged in northern Europe in 2006, although the origin and route of introduction of the particular strain of BTV-8 remain uncertain [2•]. BTV-8 spread rapidly throughout northern Europe in species of *Culicoides* midge not previously known to be efficient vectors of BTV. After an intensive vaccination campaign against BTV-8, France and adjacent countries of northern Europe were declared free of BTV as of December 2012. France also was declared free of BTV-1, which spread from the Mediterranean region to northern France in 2007. However, surveillance has confirmed that BTV-8 has continued to circulate 'silently' among livestock in France [19,20,21•]. Interestingly, although the original (2006) and recent (2015) strains of BTV-8 are genetically similar or identical, recent infections have invariably been subclinical, whereas clinical disease was common among ruminant livestock (including cattle) infected during the original outbreak.
2 *BTV-4 in France*. BTV-4 has been detected frequently in the Mediterranean region since 1998, and again appeared in Corsica in 2016, likely as a result of spread from adjacent infected Mediterranean countries where the virus continues to circulate [22]. A genetically distinct reassortant strain of BTV-4 that was identified in the Balkan countries (2014), mainland Italy (2014–2016), and Sardinia (2016–17) was identified recently in a healthy calf in mainland France (adjacent to the Swiss border) [23].
3 *BTV-3 and BTV-6 in the Mediterranean Basin*. More than 60 years after its original and only detection in Cyprus, BTV-3 reappeared in the Mediterranean Basin causing disease among sheep in Israel in 2013 and 2016–17. Similar strains of BTV-3 were also identified in Tunisia and Italy, consistent with spread of the virus via infective vectors or illegal animal movement from North Africa to Italy [24•]. These strains of BTV-3 were associated with typical signs of bluetongue among sheep in both regions, but not in cattle. In contrast, BTV-6 also reemerged in Israel in 2017 but, unlike BTV-3, BTV-6 infection resulted in clinical disease in both cattle and sheep throughout most of Israel (G Savini, unpublished data).
4 *BTV-3 in the United States*. BTV-10, 11, 13, and 17 have long been endemic in much of the United States, whereas BTV-2 is limited to the Southeastern United States and, recently, California [2•,7•,13]. Although some 11 previously exotic serotypes of BTV have invaded the Southeastern United States from the Caribbean Basin episystem since 1999, most of these viruses have not spread far from their original site of incursion. In contrast, BTV-3 has spread widely since 2006, and it is now clearly evident that the emergent strains of BTV-3 have extensively reassorted genes with those of the traditional endemic serotypes (BTV-10, 11, 13,17).
5 *Live-attenuated BTV-14 vaccine in Europe*. At least 3 (BTV-6, BTV-11, BTV-14) different South African live-attenuated BTV vaccines have been disseminated by vectors among livestock in Europe, most recently BTV-14 in western Russia, Poland, Lithuania and Spain (in a single animal imported from Lithuania) [25,26,27•]. It remains uncertain how these viruses were introduced into Europe as they were not used in official vaccination campaigns. The natural spread of live-attenuated BTV vaccine viruses also occurs elsewhere in the world, and genetic analyses confirm that these vaccine BTVs can reassort their genes with those of other endemic BTVs to create genetically distinct progeny, potentially with novel biological properties [2•,28•].

Identification of novel, small ruminant adapted BTVs

Several new serotypes of BTV have been described recently in Europe, Asia and Africa. These viruses represent a novel group of BTVs that differ in their biological and genetic properties from the historical 24 serotypes (BTV 1–24) that previously have been identified globally. Specifically, these novel BTVs exhibit minimal virulence or pathogenicity to ruminants and have all been isolated from apparently healthy goats or sheep. Some of these viruses can be transmitted by contact (horizontally) without requirement for vector insects, and some appear to cause persistent infections in their animal hosts. Although little is known about these new BTV types, some have already been classified as additional serotypes (specifically BTV 25–27), whereas others have yet to be assigned a numerical serotype designation. Serological classification of some of

Figure 1



Recently described changes in the global distribution or biology of bluetongue virus (BTV) depicting affected countries (not the precise distribution of each virus). **(a)** 'Traditional' serotypes of BTV (1–24) in Europe and the Mediterranean Basin (additional serotypes circulating are noted directly on the affected areas). Adapated from a map showing restriction zones for bluetongue virus in the European Union as of May 30, 2018 (available at http://www.bluetonguevirus.org/sites/blue/files/quick_media/Current%20EU%20BTV%20Restriction%20Zones.jpg). **(b)** Spread of BTV-3 from the southeastern United States. **(c)** Identification of new serotypes (BTV 25–27) and related novel viruses (BTV X, Y and SP) in Europe, Africa and the Middle East (BTV XJ1407 from China is not shown).

these novel viruses is complicated because of their resistance to propagation in cell culture.

The first of these novel BTVs was named Toggenburg Orbivirus after the region of its first detection in goats in Switzerland. Sequence analyses confirmed the virus to be a new BTV serotype, namely BTV-25 [29,30]. Although first detected in 2008, retrospective analyses confirmed positive samples from Swiss goats dating back to 1998 indicating that BTV-25 has circulated in that part of Europe for at least a decade and likely considerably longer. BTV-25 produces subclinical infections in goats and is only mildly pathogenic to sheep. Although BTV-25 infection is highly prevalent among goats in certain areas of Switzerland, infection was not found in either sheep or cattle cohabiting with virus-positive goats, nor in wild

animals, suggesting a highly restricted host range [31]. The high prevalence of BTV-25 infection in goats likely reflects its ability to be transmitted horizontally and induce persistent infection in this species. Experimental studies are difficult with BTV-25 because the virus is yet to be propagated *in vitro* using embryonated chicken eggs (ECE) or cell culture systems that are reliably used for propagation of BTV 1–24 [32]. A probable variant of BTV-25, BTV-Z ITA2017, was recently identified in a goat flock from North Western Italy [33•]. Like BTV-25, ruminants infected with BTV-Z ITA2017 showed no clinical signs and only low levels of RNA-emia.

Another novel BTV, BTV-26, was first isolated in 2010 from the blood of a sheep in Kuwait [34]. Blood samples were positive by BTV group-specific RT-PCR, but consistently negative in serotype-specific RT-PCR assays, indicating the presence of a novel BTV serotype. The sequence of the serotype-specific VP2 gene of BTV-26 differs from other known serotypes (BTV 1–25), but is more closely related to BTV-25 than other serotypes. Unlike BTV-25, BTV-26 can be propagated *in vitro* although its growth capability is limited to mammalian cell lines and the virus does not replicate in culicoides-derived cells (Kc). Experimental BTV-26 infection results in a higher and longer-lasting RNA-emia in goats than in sheep. Whereas BTV-26 infection is subclinical in goats, the virus causes mild disease in sheep [35,36]. BTV-26 can be spread between goats by direct contact transmission via either nasal or ocular secretions. Serological evidence of BTV-26 infection subsequently was shown among cattle and dromedaries in Mauritania and in Libyan sheep [37].

A further novel BTV serotype, now designated BTV-27, was first detected among goats in Corsica in 2014 [38]. The infected goats were positive by BTV group-specific PCR but negative in serotype-specific RT-PCR assays to each of the other 26 BTV serotypes. Sequence analyses confirm high identity with both BTV-25 and BTV-26; however, BTV-27 is not neutralized by antisera raised against other BTV serotypes. Like BTV-26, *in vitro* propagation of BTV-27 is limited to selected cell lines; specifically, the virus does not grow in ECE or in cell cultures (e.g. Kc, Vero and BHK) that are commonly used for isolation of 'traditional' BTV serotypes. Several genetically related but distinct strains of BTV-27 have been identified in healthy goats, whereas cattle appear to be refractory to infection. Like other small ruminant adapted BTVs, BTV-27 can be spread horizontally by direct contact transmission [39].

A number of other genetically distinct BTVs recently have been identified that likely represent additional virus serotypes. These include a virus designated X ITL2015 from healthy goats in Italy [40]. Although clearly a BTV in terms of reaction with group-specific RT-PCR and BTV-group specific serological testing of infected animals, the virus is virologically and serologically distinct from BTV 1–27 and is genetically most similar to recently isolated strains of BTV-27 from Corsica and to another recently discovered BTV strain (XJ1407) from China. Efforts to date to isolate X ITL2015 using ECE or cell cultures have been unsuccessful. The virus designated XJ1407 was first detected in clinically normal goats and sheep in China [41•]. Although it is most related to BTV X ITL2015, and less so to BTV-25 and 27, XJ1407 can be propagated in ECE and several mammalian and insect cell lines.

Sheeppox vaccine-derived BTV (SPV BTV) was identified in 2014 in batches of a sheeppox vaccine used in Israel [42]. This virus is closely related to BTV-26 and, like BTV-26, the virus is easily propagated in ECE and several mammalian cell lines. A genetically similar virus (BTV-Y TUN2017) was identified among healthy sheep in North Africa (Tunisia) [24•]. Unlike SPV BTV, however, BTV-Y TUN2017 does not grow in ECE or cell culture. SPV and related BTVs are only distantly related to BTV 1-27, and serum from BTV-Y TUN2017 infected sheep failed to neutralize other BTV serotypes, suggesting that it is a novel serotype. As the small ruminant adapted BTVs cause subclinical or very mild infections and their presence currently does not lead to restrictions on animal movement or trade (in contrast to those associated with the presence of BTV 1–24), these viruses currently are not problematic to the livestock industries of countries in which they are endemic. However, the biological characteristics of the newly recognized BTV serotypes are remarkably different from those of the 'classical' serotypes, and reassortment events between them could potentially result in progeny viruses with different properties [40,42,43].

BTV infections of dogs

The initial description of BTV infection of dogs resulted from problems associated with the widespread use in the United States of a live-attenuated canine vaccine contaminated with BTV-11 [44,45]. More recent canine BTV-11 infections in the United States were not associated with the use of contaminated vaccines, and the canine viruses were genetically similar to those circulating in livestock at the same time in the same area [46,47]. Similarly, BTV infection of dogs is prevalent in North Africa, and is associated with neither contaminated canine vaccines nor the ingestion of BTV-infected meat [48]. It is assumed, therefore, that dogs may become infected through the bites of BTV-infected vector midges, as also has recently been proposed for related African horse sickness virus infections of dogs in South Africa [49]. BTV-11 infection is especially devastating in pregnant bitches, leading to abortion and death of the affected bitch.

The phenomenon of BTV over-wintering

Infection with traditional BTV serotypes (BTV 1–24) is seasonal in temperate regions of the world, reflecting the abundance of vector midges during late summer and autumn. How BTV persists between seasons in such

regions has been a topic of considerable controversy as transovarial transmission of virus has not been described in *Culicoides* spp. [50]. Recent studies in BTV-endemic temperate regions confirm the presence of low numbers of BTV-infected adult midges during winter months, suggesting that BTV can over-winter in long-lived vectors [51–53]. Of particular relevance is the fact that BTV-infected midges display profoundly altered feeding behavior that results in markedly reduced attraction to the light traps that have long been the mainstay of vector surveillance for BTV. As a result, exclusive use of light traps for vector surveillance can result in serious errors in determination of the regional risk of BTV transmission, and failure to detect the presence of BTV-infected, over-wintering midges [54•].

Epizootic hemorrhagic disease

Epizootic hemorrhagic disease (EHD) is a significant cause of both morbidity and mortality in North American white-tailed deer and certain other species of wild ruminants whereas, with the notable exception of Ibaraki virus, EHDV infections of ruminant livestock are typically subclinical [1•,3]. There are 7 serotypes of EHDV recognized currently, and Ibaraki virus, which is the cause of sporadic disease outbreaks in cattle in Asia, is a representative of serotype 2 (EHDV-2). Although the global distribution and epidemiology of BTV and EHDV infections are similar, EHDV infections have been less intensively studied because they are of less significance to animal agriculture as compared to those caused by BTV, particularly in regards to restrictive non-tariff trade barriers imposed on livestock from BTV endemic areas. Although most natural and experimental EHDV infections (other than with Ibaraki virus) of livestock are subclinical or asymptomatic, outbreaks of EHD recently have been reported among cattle in the Mediterranean Basin, Reunion Island, South Africa, the United States, and elsewhere [1•,3,16,55–57]. It is clearly apparent that certain strains of EHDV are more virulent to cattle than others, notably Ibaraki virus (EHDV-2) and the recently described strains of EHDV-2, 6, and 7 that caused disease among cattle in several countries. Like BTV, however, the molecular basis of EHDV virulence is uncharacterized as other strains of these same EHDV serotypes (EHDV-2,6,7) typically do not cause significant disease among cattle in other endemic regions of the world. Similarly, whole genome sequence comparisons showed no obvious differences in the strains of EHDV 1 and 2 associated with disease outbreaks among cattle in the Midwestern United States and viruses from apparently unaffected animals in the same region [7•,8] (WC Wilson, unpublished data). The role of host and the related environmental factors in precipitating outbreaks of EHD in cattle are poorly defined.

There is considerable genetic variation among the strains of EHDV that circulate within endemic regions, including virus strains of the same serotype. For example, at least three different serotypes of EHDV currently circulate in North America, one of which (EHDV-6) is a relatively recently introduced serotype. EHDV-6 spread into the Southeastern United States from the adjacent Caribbean ecosystem but, like BTV-3, only strains of EHDV-6 that include reassorted genes from the historically endemic EHDVs (EHDV-1,2) were able to expand their range beyond the southeastern United States.

Lessons learned from the global emergence/re-emergence of BTV and EHDV

1 It has long been speculated that climate change will lead to expansion of the global range of specific arboviruses and their associated diseases, but recent changes in the global distribution and impacts of both BTV and EHDV infections of ruminants have been especially dramatic. Not only has climate change apparently lead to changes in the dynamics of specific vectors, but it also has been associated with the transition of indigenous midges not thought to be virus vectors into ones that efficiently serve as biological vectors of BTV in particular.

2 The enormous genetic diversity of these orbiviruses (including anthropogenic influences such as use of live-attenuated vaccines) can lead to the emergence of viruses with unique biological properties, including the capacity for horizontal and vertical (transplacental) transmission and spread to other species (e.g. dogs). Reassortment of the genes of incursional orbiviruses with those already present (endemic) viruses has facilitated the successful introduction and spread of novel reassortant progeny between episystems.

3 Live-attenuated orbivirus vaccines clearly have serious inherent limitations, including their capacity for spread by vector midges, reassortment with endemic viruses to create genetically novel progeny, and expression of novel properties including transplacental transmission, transmission to heterologous species, etc. [2•,28•,58,59].

4 Novel vaccination strategies employing safe and effective DIVA-compatible vaccines will be needed to limit the future spread and economic impact of these re-emerging orbivirus infections of ruminants [2•,28•,60–62].

Conflicts of interest statement

Nothing declared.

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